THE RIBOFLAVIN CONTENT OF MEATS[1]

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THREE FIGURES

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Since the demonstration that riboflavin is one of the components of the vitamin B complex (György, Kuhn and Wagner-Jauregg, '34 a) interest has been stimulated in the distribution of this substance in animal tissues. Determinations of the 'vitamin G' content of such tissues have been reported (Aykroyd and Roscoe, '29; Hoagland and Snider, '30; Day, '31; Roscoe, '33; György et al., '34; Christensen et al., '36; Kellogg, '36), but one cannot be certain that all of these determinations measured riboflavin.

In general, two methods have been developed for the quantitative estimation of riboflavin. The first of these consists of extracting the material with an organic solvent and comparing the depth of color of the extract with a standard solution of pure riboflavin or some similarly colored substance previously standardized against riboflavin. von Euler and Adler ('34) and Charite and Khaustov ('35) have utilized this technic as a means of measuring the flavin content of animal tissues.

The second general method consists of estimating the amount of flavin in a substance from the growth induced in standard test animals. Since this would measure the content

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of biologically active flavin, it would appear to be more applicable to nutritional studies. This method has been used by György ('35) and Carlsson and Sherman ('38) to study natural products of animal origin. Several independent investigators have reported that the 'vitamin G' as measured by the Bourquin-Sherman method ('31) is flavin (Booher, Blodgett and Page, '34; Bisbey and Sherman, '35; Copping, '36; Levine and Remington, '37).

EXPERIMENTAL METHOD

Biological assays have been made of the riboflavin content of beef brisket, lamb chops, bacon, cured and fresh ham and pork liver. The flavin-deficient diet and experimental technic have been adequately described in detail (Day and Darby, '36, '36 a; Day, Darby and Langston, '37). Two casein preparations were used in the experimental diets. They were 'casein, edible, muriatic,'[2] extracted with 60% ethyl alcohol in this laboratory according to the method of Sherman and Spohn ('23) and a 'vitamin-free' casein.[3] These two preparations have been shown to be essentially flavin-free by biological tests and by examination of eluates from them under ultraviolet light (Day and Darby, '37).

The fresh meats were purchased twice a week from local retail markets, and were stored in a refrigerator until used. The uncooked meat supplements to the flavin-deficient diet were weighed out on an analytical balance and fed at once in porcelain ointment pots. The meats were consumed immediately and completely by all the animals except those receiving the largest supplements of bacon. Each meat was fed at three levels to groups of rats under properly controlled conditions. The amounts of supplements indicated in table 1 were fed six times a week, except the pork liver. This was given in twice the indicated quantities three times a week. Only the lean portions of the ham, lamb and beef brisket were fed.

[2] Manufactured by the Casein Company of America, 350 Madison Avenue, New York.

[3] 'Labco' brand, manufactured by the Casein Company of America.

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TABLE 1

Data regarding growth and incidence of cataract in rats receiving a riboflavin-deficient diet supplemented with various amounts of meats, and the amount of riboflavin contained in these meats as computed from growth data

SUPPLEMENT [1]	NUMBER OF RATS	NUMBER SHOWING CATARACT	AVERAGE GAIN [2]	RIBOFLAVIN	
			gm.	*units per gram* [3]	*micrograms per gram* [4]
Controls [5]	5	3	—6.2		
Beef brisket, 1.0 gm.	4	0	12.2		
Beef brisket, 2.0 gm.	5	0	43.0	0.74	1.9
Beef brisket, 4.0 gm.	5	0	78.6		
Controls	8	5	—4.9		
Lamb chops, 0.5 gm.	10	2	10.6		
Lamb chops, 1.0 gm.	10	1	27.1	1.1	2.8
Lamb chops, 2.0 gm.	9	0	56.9		
Controls	9	6	—2.8		
Bacon, 1.0 gm.[6]	9	4	5.6		
Bacon, 2.0 gm.[6]	7	0	7.7	0.36	0.9
Bacon, 4.0 gm.[6]	2	0	14.5		
Controls	10	5	—2.5		
Ham, cured, 1.0 gm.	10	3	14.8		
Ham, cured, 2.0 gm.	10	0	39.6	0.81	2.0
Ham, cured, 4.0 gm.	10	0	93.6		
Controls	10	5	—4.9		
Ham, fresh, 0.5 gm.	10	4	15.7		
Ham, fresh, 1.0 gm.	10	0	27.0	1.2	3.0
Ham, fresh, 2.0 gm.	9	0	63.5		
Controls	8	4	—6.1		
Pork liver, 0.05 gm.	10	3	7.6		
Pork liver, 0.1 gm.	10	0	22.4	9.2	23.0
Pork liver, 0.2 gm.	9	0	57.9		

[1] The amount of fresh meat fed six times a week.

[2] Gain in the 8 weeks during which the rats received meat supplements.

[3] One unit is the amount of riboflavin (vitamin G) required to induce a mean growth of 24 gm. in 8 weeks, when fed six times a week.

[4] Expressed in terms of the biologically equivalent amount of crystalline riboflavin.

[5] There was a total of forty-eight control rats used in the series. Of these, twenty-five (52%) developed cataract during the 10-week period, but thirty-two (67%) exhibited cataract before death.

[6] The data on animals receiving bacon are for an assay period of 4 weeks.

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It was impossible to eliminate entirely the fat and connective tissue from the samples of brisket to be fed. The bacon fed was sliced, rindless and of average fatness. The samples of bacon weighed out contained lean and fat in approximately the same ratio as they were found in the product as purchased. The high fat content of the bacon appeared to injure

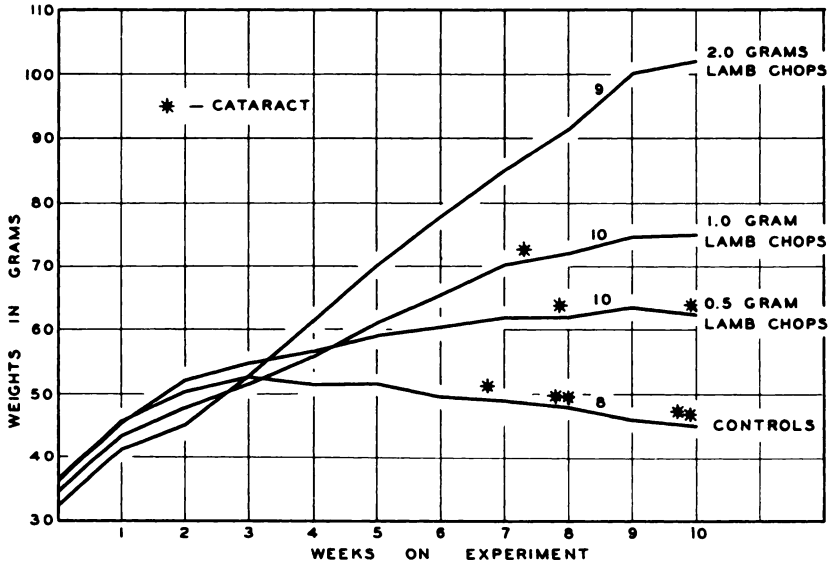

Fig. 1 Average growth curves of groups of rats that received a flavin-deficient diet supplemented with fresh lamb. The rats received only the flavin-deficient diet for the first 2 weeks, and thereafter each animal received the amount of lamb indicated at the end of the curve, six times a week. The numbers on the curves indicate the number of rats, and the asterisks mark the time of appearance of cataract in individual animals.

the animals, and many of the rats receiving bacon died before the end of the experiment. Consequently, the bacon data have been presented for a 4-week period.

RESULTS

Figures 1 and 2 give mean weight curves of groups of rats which received various amounts of fresh lamb and pork liver. The rats were given only the riboflavin-deficient diet for the

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first 2 weeks, after which they received the supplements of fresh meat six times a week that are indicated on the curves. Similar data were obtained for the other meats studied, but to conserve space the essential facts are presented briefly in table 1. The mean weights of the groups of rats, when they were started on the experimental diet at 21 days of age, were between 32.6 and 42.3 gm. When the feeding of meats was

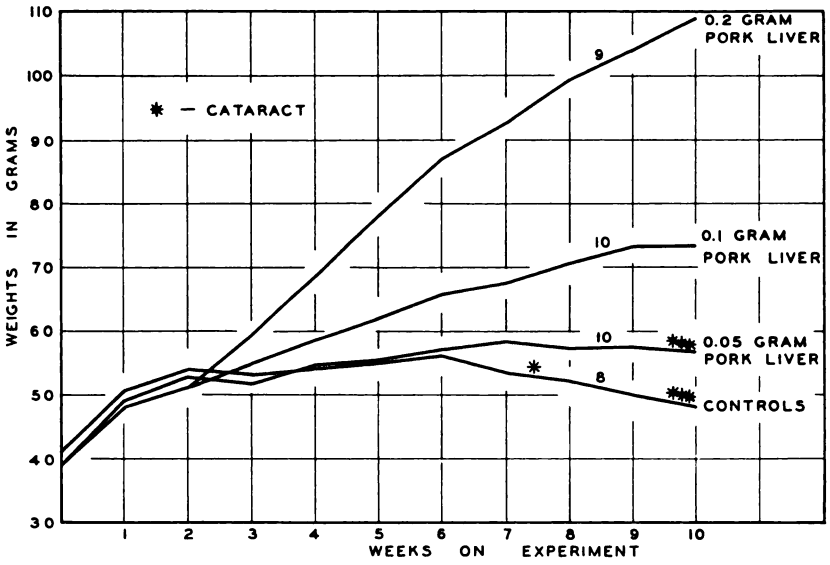

Fig. 2 Growth curves of groups of rats that received a flavin-deficient diet supplemented with fresh uncooked pork liver. The rats received only the deficient diet for the first 2 weeks, and thereafter each animal received the amount of liver indicated at the end of the curve, six times a week. The numbers on the curves indicate the number of rats, and the asterisks mark the time of appearance of cataract in individual animals.

started (2 weeks later) the mean weights of the groups were between 43.6 and 54.0 gm. The animals were fed the meat supplements for a period of 8 weeks and the average net gain for each group is given in the table.

Published experiments (Day, Darby and Langston, '37) have shown that our method of 'vitamin G' assay measures riboflavin, at least when the growth is 10 gm. weekly or less.

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The content of riboflavin in the meats studied has been computed from growth data and presented in two ways (table 1). One column gives the number of units of riboflavin per gram of meat. We have employed this unit to mean the amount of riboflavin required, when fed six times a week to depleted animals, to promote an average net gain of 24 gm. during the 8 weeks that the rats were fed the supplements. The meats assayed were found to contain the following amounts of riboflavin (vitamin G): beef brisket, 0.74; lamb chops, 1.1; bacon,

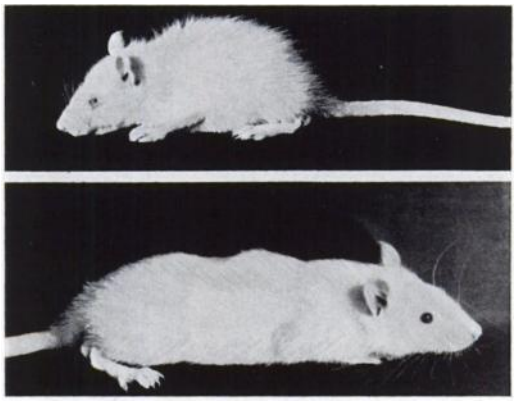

Fig. 3 Photographs of typical experimental animals. Above, negative control showing alopecia of the eye-lid and cataract. Below, animal which received 2.0 gm. fresh ham six times weekly. No cataract or alopecia was evident in this animal at any time. The rats were photographed on the fifty-ninth day on experiment, at which time they weighed 37 and 101 gm. respectively.

0.36; cured ham, 0.81; fresh ham, 1.2, and pork liver, 9.2 units per gram.

With our experimental diet, technic and strain of rats, one unit as defined above is equal to approximately 2.5 micrograms of crystalline riboflavin (Day, Darby and Langston, '37, and subsequent unpublished experiments). It therefore becomes a simple matter to translate units into the equivalent number of micrograms of riboflavin. The last column in table 1 gives the content of riboflavin in the meats studied computed in this way. To be sure, the flavin may not all have

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existed in the organ or tissue as simple riboflavin; at least a part of it may have been riboflavin phosphate or a flavoprotein complex. The computed content of riboflavin must be interpreted as meaning that the meat contained a total amount of riboflavin—free and combined—biologically equivalent to the amount of riboflavin stated. Thus, 1 gm. of pork liver contained a total amount of flavin nutritionally equivalent (for the rat) to 23 micrograms of riboflavin.

DISCUSSION

Reports from several laboratories (Daniel and Munsell, '37) indicate that beef muscle (round steak) contains between 1.0 and 1.25 units of 'vitamin G' per gram of fresh meat. The lower content of riboflavin in brisket (0.74 unit per gram), as found in this investigation, is probably due to the greater amount of connective tissue and fat in this cut of meat.

Christensen, Latzke and Hopper ('36) reported that fresh pork contained more than 1.0 unit of vitamin G per gram. This agrees very well with our value of 1.2 units of flavin per gram of fresh ham (pork). The decidedly lower content of riboflavin in bacon is readily explained upon the basis of the high fat content of this pork product.

Our results indicating that lamb muscle contains 1.1 units per gram demonstrates that this meat is essentially the equivalent of beef muscle and pork muscle as a source of riboflavin in the diet. It is apparent that muscle meats from pork, beef and lamb are surprisingly similar in their content of riboflavin, each containing slightly more than 1 unit per gram.

Although data on the riboflavin content of pork liver have not previously been available, the livers of both beef and veal were found to contain approximately 10 units of vitamin G per gram (Day, '31). The content of 9.2 units per gram for pork liver, as here reported, indicates that liver from this species is essentially equivalent in flavin content. As is true in the case of muscle meats, the livers of market beef, veal and pork are surprisingly similar in their content of riboflavin.

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Fresh liver, whether from beef, veal or pork, is the equivalent of many samples of dried yeast (Daniel and Munsell, '37), and on the basis of dry weight is apparently the richest known natural food source of riboflavin.

A total of forty-eight control rats was used in the series of experiments. Although the animals receiving meat supplements were killed at the end of 10 weeks, the control rats receiving only the deficient diet were retained until death. Of these, twenty-five (52%) exhibited cataract by ophthalmoscopic examination during the 10-week experimental period, but thirty-two (67%) exhibited cataract before death. The average time of appearance of cataract was 65 days, and the average survival period was 88 days.

A perusal of table 1 reveals the interesting fact that cataract appeared in the groups of animals receiving small amounts of meats, although the incidence was lower than among the negative controls. Cataract was found only rarely in the groups of animals receiving the larger amounts of meat. This is in complete agreement with our previously published statement that rats receiving a limited amount of flavin only occasionally developed cataract where the growth was 30 gm. or more in the 10-week experiment, and in no case developed cataract where the net growth was more than 56 gm. in 10 weeks (Day and Darby, '36 a). This inverse relation between riboflavin intake and incidence of cataract is exactly what would be expected in the light of our recent experiments showing that the cataract resulting from 'vitamin G' deficiency may be prevented by the feeding of pure riboflavin (Day, Darby and Langston, '37), and in its early stages can be arrested by the administration of pure riboflavin (Day, Darby and Cosgrove, '38).

SUMMARY

Using a rat-growth method, determinations of the riboflavin content of a number of meats have been made. The organs and tissues studied were found to contain the following amounts of riboflavin, expressed in units per gram of fresh

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uncooked material as purchased: beef brisket, 0.74; lamb chops, 1.1; bacon, 0.36; cured ham, 0.81; fresh ham, 1.2 and pork liver, 9.2.

With our diet, technic and strain of animals, unpublished experiments indicate that 1 unit is equivalent to approximately 2.5 micrograms of crystalline riboflavin. The meats assayed were therefore found to contain amounts of 'vitamin G' biologically equivalent to crystalline riboflavin as follows: beef brisket, 1.9; lamb chops, 2.8; bacon, 0.9; cured ham, 2.0; fresh ham, 3.0 and pork liver, 23 micrograms per gram.

Of a total of forty-eight controls receiving the deficient diet only, twenty-five (52%) developed cataract during the 10-week experiment, and thirty-two (67%) exhibited cataract before death. The incidence of cataract in the groups of rats receiving meat supplements varied in inverse relation to the growth.

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